14. Q. S. Mayer and R.E. Shoup, J. Chromatogr., **255**, 533 (1983).
15. C. J. Morales-Olivas, H. Bedale, R. Bello, and E. Rubio, International Congress of Pharmacology, 7th edition, Abstracts, Paris (1978), p. 778.
16. M. Palkovits, Brain Res., **59**, 449 (1973).
17. T. Yamamoto and S. Ueki, Physiol. Behav., **19**, 105 (1977).

MOLECULAR MECHANISMS OF DEVELOPMENT OF CARDIOVASCULAR PATHOLOGY

L. T. Malaya,* T. Yu. Shchegoleva, and L. K. Bakhova

UDC 616.1-092:577.2

KEY WORDS: microwave dielectrometry; millimeter waveband; adenylate cyclase system; essential hypertension; ischemic heart disease

The study of the complex pattern of biochemical changes at different stages of development of cardiovascular pathology reflects considerable metabolic disorders in the body. Among the causes of their appearance, a leading place is occupied by imbalance of the neuroendocrine systems [5]. All lesions must be manifested to some degree or other at the membrane level. This is the basis for a number of tests, for example, a raised blood enzyme level, when many tissue enzymes are found in the blood as evidence of serious tissue damage with cell destruction. In the native cell these changes must be determined by dysfunction of membrane mechanisms. The study of the mechanisms of intracellular warning, based evidently on functional responses of the cell, which are evidently universal [2], and effected through regulatory action, constitutes an important problem. Considerable interest of investigators has been attracted to the study of the regulatory function of the blood cells, by the use of delicate biochemical and biophysical methods. Some workers [1] observed stable differences of the dielectric constant of suspensions of blood cells in different forms of cardiovascular pathology. Pathological changes in ischemic heart disease (IHD) in stage II of essential hypertension (EH II), and in borderline hypertension (BH) are manifested at the cellular level by a difference in values of the complex dielectric constant of an erythrocyte suspension at a wavelength of λ = 7.6 mm. The molecular mechanisms responsible for these differences must probably be associated with catecholamine function in the adenylate cyclase system (ACS) of the cell.

The aim of this investigation was to study responses of the cell to procedures targeted on components of the ACS.

EXPERIMENTAL METHOD

Experiments were carried out on erythrocytes from healthy donors and patients with IHD, EH II, and BH. The value measured was the dielectric constant within the region of dispersion of free water (λ = 7.6 mm). Depending on the change in this parameter, the presence or absence of response of cell systems to factors acting on the protein components of ACS of the erythrocytes was established. The dielectric constant was measured on a microwave dielectrometer, specially modified for these purposes [6]. The error of measurement was 3% relative to

*Academician of the Russian Academy of Medical Sciences.

Research Institute of Internal Medicine, Ministry of Health of the Ukraine. Institute of Radiophysics and Electronics, Academy of Sciences of the Ukraine, Khar'kov. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 572-574, June, 1992. Original article submitted November 6, 1991.

 0007-4888/92/0006-0762$12.50

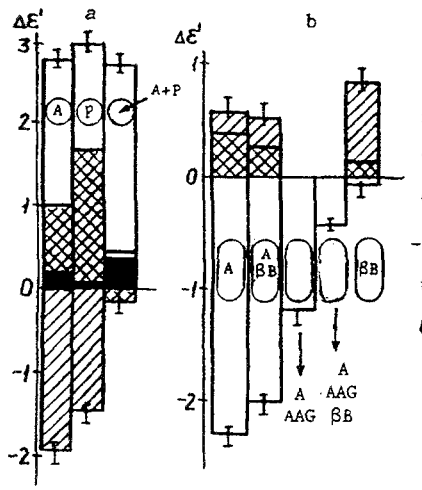

Fig. 1

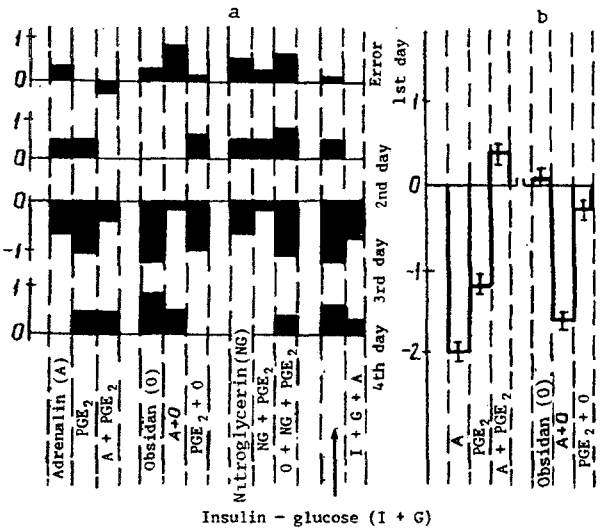

Fig. 2

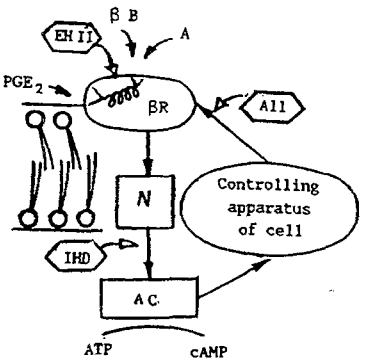

Fig. 3

Fig. 1. Changes in real part of dielectric constant of erythrocyte suspension under normal and pathological conditions, with procedures targeted on components of ACS. A) Adrenalin; P) prostaglandin E_2; B) blocker; AA) ascorbic acid; G) reduced glutathione. a: Unshaded columns – EH II 1; cross-hatched columns EH II 2; columns shaded black – IHD; oblique shading – normal. b: Cross-hatched columns – allergic state; obliquely shaded – IHD; unshaded columns – normal state.

Fig. 2. Changes in real part of dielectric constant of erythrocyte suspension as a result of procedures acting on β-receptor and its membrane environment during development of acute myocardial infarction. a) Acute myocardial infarct; b) control (healthy individuals).

Fig. 3. Diagram showing control of β-receptor in ACS and possible sites of lesion during development of cardiovascular pathology. All) allergic state.

ε', ε'', and the error of measurement of changes was 1%. The volume of the erythrocyte suspension poured into the cuvette did not exceed 0.01 ml. The time taken to measure one sample was 2-3 min. The erythrocyte suspension was reduced to a single concentration according to the reading of the photoelectric colorimeter (λ_1 = 580 nm). The agents used were as follows: adrenalin (from "Sigma," USA; A, 10^{-5} M), prostaglandin E_2 (Tallinn, PGE_2, 10^{-8} M); propranolol (Obsidan, O, 10^{-4} M); ascorbic acid (AA, $4.4 \cdot 10^{-4}$ M); glutathione ($3 \cdot 10^{-3}$ M); nitroglycerin ($0.8 \cdot 10^{-5}$ M); insulin (I, 0.08 mU/ml); glucose (G, 10^{-2} M). Values of ε^* are given in conventional units.

EXPERIMENTAL RESULTS

Disturbances in ACS during pathology are clearly visible as a change in ε' in the cell response to alternative regulators of the system, namely adrenalin and prostaglandin E_2. The effect of protection by PGE_2 against the action of A, which we studied in detail previously [3], under normal conditions is essentially variable with differences in the intensity of the changes in the cardiovascular system (Fig. 1a). Moreover, patients with EH II (Fig. 1a) can be subdivided on the basis of this parameter into two groups, with the presence of a protective effect, but with distortion compared with the normal response to A and PGE_2, and with absence of protection. These groups differ in their clinical features. In IHD the response of the system to A and PGE_2 is the weakest possible, but the protective effect is just as weak. In stable angina, a different protection factor may perhaps be realized.

In relation to binding sites of PGE_2 and A (according to previous data we consider that these are two different sites [5]) several conclusions can be drawn on the basis of a combination of factors with action on and blocking the β-receptor, as well as influences on its membrane environment. In this case, for instance, nitroglycerin has a marked effect on the action of PGE_2, but virtually no effect on the action of adrenalin, propranolol, and a combination of the two. The PGE_2 site may perhaps be connected conformationally with the lipid bilayer of the

membrane, whereas the adrenalin site may be independent of the lipid matrix within certain limits. If regulation is disturbed, the link between the spatially distant fragments of the receptor macromolecule may be modified, as a result of insertion of a nonspecific regulator into it, such as, for example, Ca^{2+}, cholesterol, and so on. Blockade of the receptor sites is ruled out, because the response of the cell systems takes place in all types of pathology.

It is interesting to note that the β-receptor blocker did not affect the action of PGE_2 in pathological states (normally their action is opposite in phase). Propranolol blocks adrenalin receptors in three ways. Normally by incomplete protection, regulated by feedback through ascorbic acid and glutathione, which evidently determines the role of this pair as a universal reducing system of the cell; in IHD it is very weak protection by the blocker; in allergic states it is complete protection, when, it would seem, feedback is ruled out (Fig. 1b).

Diagrams representing action on the β-receptor in ACS during the development of acute myocardial infarction, complicated on the 3rd day by an allergic state, are shown in Fig. 2. The development of an acute infarct was manifested as a general weakening of the cell response (Fig. 2b, normal). Hyperthermia, arising on the 3rd day in the patient, significantly activated the cell response, perhaps through the exclusion of feedback. After discontinuation of hyperthermia the molecular mechanism of alternative regulation by PGE_2 and A returned to its previous weakly active state, characteristic of stable angina.

The effects observed can be represented by a diagram of regulation of the β-receptor in ACS, on which we can identify perfectly concrete sites that are subject to attack by pathological agents. The possible sites for disturbances of the ACS control system in different forms of pathology are shown in Fig. 3.

These views correlate well with the biochemical parameters, although they have an essential advantage. Work with native cells enables the reversibility of the lesions to be assessed for a particular individual and, in case reversibility exists, it enables the factors restoring the regulatory system to be deliberately chosen. Tracking and predicting clinical manifestations during development of cardiovascular pathology is also a direct possibility.

LITERATURE CITED

1. V. P. Veselovskii, V. F. Bogoyavlenskii, V. N. Kryukov, et al., Inventor's Certificate No. 667200 USSR.
2. V. L. Voeikov, Progress in Science and Technology. Bioorganic Chemistry [in Russian], Vol. 2, Moscow (1984), p. 472.
3. L. T. Malaya, T. Yu. Shegoleva, and L. K. Bakhova, Fiziol. Zh. (Kiev), **33**, No. 1, 96 (1987).
4. L. T. Malaya, T. Yu. Shegoleva, and L. K. Bakhova, Biofizika, No. 4, 629 (1988).
5. T. V. Fetisova and R. A. Frol'kis, Biochemistry of Myocardial Infarction [in Russian], Moscow (1976), p. 168.
6. T. Yu. Shegoleva, Radiotekh. Élektron., No. 2, 23 (1981).